AR201-13309

VELSICOL
CHEMICAL CORPORATION

November 16, 2001

Administrator Christie Whitman
US Environmental Protection Agency
Attn: Chemical Right to Know Program
PO Box 1473
Merrifield, VA 22116

RE: HPV Test Plan and Robust Summary for Triethylene Glycol Dibenzoate

Dear Ms. Whitman:

Velsicol Chemical Corporation (Sponsor 899 and Registration :) is committed to participation in the High Production Volume Chemical Challenge Program. As such, I am pleased to submit the Robust Summary for Triethylene Glycol Dibenzoate CASRN: 120-56-9. We have submitted the summary as a Word document and have also included a hard copy. The test endpoints recommended under the HPV Challenge program have been completed and summarized. It is our recommendation that no further testing for this chemical is necessary.

Please contact me at 847-635-3454 if you have comments or questions regarding this submission.

Sincerely,

Emily Clark
Regulatory Compliance Specialist

Enclosure

MR-52957

AR201-13309A

Triethylene Glycol Dibenzoate
Robust Summary

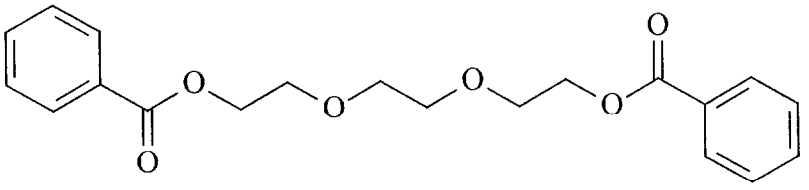
Triethylene glycol dibenzoate is a high solvating plasticizer used in the following applications:

- Solvator for PVC
- Vinyl flooring
- Adhesives
- Plasticizers in elastomers
- Latex caulks and sealants
- Castable polyurethanes
- Color concentrates for PVC

Synonyms:

- CASRN: 120-56-9
- Benzoflex® S-358
- Ethanol, 2,2'-[1,2-ethanediylbis(oxy)]bis-, dibenzoate
- Ethylenebis(oxyethylene) dibenzoate

The structural formula is:



Triethylene glycol dibenzoate

Triethylene glycol dibenzoate is a white solid.

Other typical properties include:

Relative Density:	1.25
Henry's Law Constant	1.8×10^{-9} atm.m^3/mol
Flash Point (PMCC)	151°C

Not explosive or pyrophoric

Reference:

Benzoflex S-358. Determination of Physico-Chemical Properties. Huntingdon Life Sciences. 1998.

TRIETHYLENE GLYCOL DIBENZOATE TEST PLAN

Study	Data Available	Data Acceptable	Testing Required
Boiling Point	Yes	Yes	No
Freezing Point	Yes	Yes	No
Vapor Pressure	Yes	Yes	No
Partition Coefficient	Yes	Yes	No
Water Solubility	Yes	Yes	No
Photodegradation	Estimation	Yes	No
Stability in Water	Estimation	Yes	No
Transport (Fugacity)	Estimation	Yes	No
Biodegradation	Yes	Yes	No
Acute Toxicity to Fish	Yes	Yes	No
Acute Toxicity to Aquatic Invertebrates	Yes	Yes	No
Acute Toxicity to Aquatic Plants	Yes	Yes	No
Acute Toxicity Oral and Dermal	Yes	Yes	No
Repeated Dose Toxicity	Yes	Yes	No
Developmental Toxicity	Yes	Yes	No
Reproductive Toxicity	Yes	Yes	No
Bacterial Reverse Mutation Assay	Yes	Yes	No
Genetic Toxicity Gene Mutation	Yes	Yes	No
Genetic Toxicity Chromosome Aberrations	Yes	Yes	No

BOILING POINT

Test Substance: Benzoflex S-358 is white solid.

NAME	CAS #	%
Triethylene Glycol Dibenzoate	120-56-9	96.9
Triethylene Glycol Monobenzoate	25022-51-7	1.26
Ethylene Glycol Monobenzoate	94-33-7	0.19
Diethylene Glycol Dibenzoate	120-55-8	0.16
Diethylene Glycol Monobenzoate	20587-61-5	0.12
Ethylene Glycol Dibenzoate	94-49-5	0.11
Unidentified Component #1	Unknown	0.16
Unidentified Component #2	Unknown	0.17

Test Method: OECD 102, EEC A1

GLP: Yes

Year Performed: 1997

Results: Melting point value 43.5-49.0°C

Data Quality: 1, Reliable without restrictions

References: Benzoflex S-358 Determination of Physical/Chemical Properties Report. Huntingdon Life Sciences. 1998.

FREEZING POINT:

Test Substance: See boiling point for purity

Test Method: OECD 103, EEC A2

GLP: Yes

Year Performed: 1997

Results: Pressure 777mmHg, decomposition yes > 230°C

Data Quality: 1, Reliable without restrictions

References: Benzoflex S-358 Determination of Physical/Chemical Properties Report. Huntingdon Life Sciences. 1998.

VAPOR PRESSURE

Test Substance: See boiling point for purity

Test Method: OECD 104, EEC A4

GLP: Yes

Year Performed: 1997

Results: 1.9×10^{-7} mm Hg @ 25°C
1.7×10^{-6} mm Hg @ 50°C
6.0×10^{-5} mm Hg @ 100°C

Data Quality: 1, Reliable without restrictions

References: Benzoflex S-358 Determination of Physical/Chemical Properties Report. Huntingdon Life Sciences. 1998.

PARTITION COEFFICIENT

Test Substance: See boiling point for purity

Test Method: OECD 117, EEC A8

GLP: Yes

Year Performed: 1997

Results: log P_{ow} = 3.2

Calculated Value: 2.7662

Measured Value: 3.2

Data Quality: 1, Reliable without restrictions

References: Benzoflex S-358 Determination of Physical/Chemical Properties Report. Huntingdon Life Sciences. 1998.

WATER SOLUBILITY

Test Substance: See boiling point for purity

Test Method: OECD 105, EEC A6

GLP: Yes

Year Performed: 1997

Results: 30.4 mg/l @ 30°C, pH = 7.0 low solubility

Data Quality: 1, Reliable without restrictions

References: Benzoflex S-358 Determination of Physical/Chemical Properties Report. Huntingdon Life Sciences. 1998.

ENVIRONMENTAL FATE

PHOTODEGRADATION

The rate constant and half-life for the atmospheric gas-phase reaction between photolytically produced hydroxyl radicals and triethylene glycol dibenzoate have been estimated using the software AOPWIN v1.88.

The rate constant for the reaction, assuming a 24-hour day and a hydroxyl radical concentration of 1.5 x 10^6 mol/cm^3, was 32.97 x 10^{-12} cm^3/mol-sec and the half-life was 0.162 days (3.893 hours).

The program did not estimate a rate constant for reaction with ozone (only olefins and acetylenes are estimated) and there were no structures matched with the experimental database.

Reliability: Estimated value based on accepted model.

Reference: Triethylene Glycol Dibenzoate. Estimation of Photodegradation Using the Atmospheric Oxidation Program (AOPWIN). Huntingdon Life Sciences. 2001.

STABILITY IN WATER

The aqueous hydrolysis rate constant and half-life for triethylene glycol dibenzoate have been estimated using the software program HYDROWIN v1.66.

The calculated rate constant for the base-catalyzed reaction (K_b) at pH>8 and at 25°C was estimated to be 1.645 x 10^{-1} L/mol-sec. Half-lives at pH 7 and 8 were estimated to be 1.335 years and 48.77 days, respectively.

The program does not calculate neutral hydrolysis rate constants. Therefore reported half-lives may be over estimates.

The fragment –CH_2- CH_2-O-R was not available in the program's library. Therefore fragment - CH_2- CH_2 –0-CH_3 was substituted. This is unlikely to have affected the estimation result significantly.

Reliability: Estimated value based on accepted model.

References: Triethylene Glycol Dibenzoate. Estimation of Hydrolysis Rate Using the HYDROWIN Program. Huntingdon Life Sciences. 2001.

TRANSPORT (FUGACITY)

The fate and behavior of triethylene glycol dibenzoate in a model environment consisting of four main components, air, water, soil and sediment, has been evaluated using the Mackay Level III Fugacity Model, version 2.10.

Results from the evaluation indicate that the distribution of triethylene glycol dibenzoate between the soil and water compartments is likely to be highly dependent on the route of entry, as movement between environmental compartments is very restricted except for deposition onto soil and water from air. Air concentrations will be small because of the low vapor pressure of triethylene glycol dibenzoate.

The main mechanisms of loss will be microbial degradation in soil and water but losses through advection by rivers and and atmospheric oxidation may also be significant. It appears probable that susceptibility to microbial degradation in both the soil and water compartments will result in low persistence of triethylene glycol dibenzoate in the environment.

Inputs to the Model:

Physical Chemistry Properties

Chemical Type	1	A chemical that partitions into all media
Molecular mass	358.39	Molecular formula $C_{20}H_{22}O_6$
Data Temperature	25°C	
Log K_{ow}	3.2	VCL273/972409
Water Solubility(g/m^3)	30.4	VCL273/972409
Vapor Pressure (Pa)	2.533E-05	VCL273/972409
Melting Point	46.25°C	VCL273/972409

Half-Lives

Half-life in Air	3.893 hours	VCL366/010079
Half-life in Water	280 hours	VCL297/983318 A factor of ten was added for this study to allow for probability of lower degradation in some other water bodies
Half-life in Soil	560 hours	Assumed twice that of water. This is in line with advice in the TGD Part II for a readily biodegradable substance with a soil-water partition coefficient of less than 100 l/kg
Half-life in Bulk Sediment	560 hours	Assumed twice that of water. This is in line with advice in the TGD Part II for a readily biodegradable substance with a soil-water partition coefficient of less than 100 l/kg
Half-life in Suspended Sediment	280 hours	No data therefore because the close proximity of the bulk water phase, the half-life for the latter was used.
Half-life in Fish	280 hours	No data therefore because the close proximity of the bulk water phase, the half-life for the latter was used.
Half-life in Aerosol	3.893 hours	No data therefore because the close proximity of the bulk air Phase, the half-life for the latter was used.

Dimensions to the Model Environment

The parameters that define the model environment are:
Volume of each environmental compartment (m^3)
Density of each environmental compartment (kg/m^3)
Organic carbon content of soil and sediments (g/g)
Lipid content (kg/m^3)
Transport velocities between compartments (m/h)

Emissions to the Model Environment

The route and magnitude of emissions to the environment will vary depending on the stage of the product life cycle being considered. Because no information was available on probable real-life emissions into the environment the recommendation of Mackay et al (1996) was followed; that is the model was run for 1000 kg/h emissions to each of the air, water and soil compartments individually and then in total. This standardized approach allows comparison with other compounds and provides information on the main source of the chemicals in each compartment. Specific properties which should be taken into account when evaluating outputs from the model are that it has a very low vapor pressure so that losses to the atmosphere during manufacture and processing will be limited and that its ready biodegradability means that concentrations in the effluent from sewage treatment plants will be very low.

Results:
Under equilibrium steady state condition (Fugacity Model Level 1) triethylene glycol dibenzoate distributed almost entirely between the soil and water compartments

Compartment	Amount
Air	0.0025%
Soil	57.62%
Water	41.05%
Sediment	1.28%

Using the Level III program and with emissions of 1000 kg/h to each of the air, water and soil compartments, the model estimated the following distribution:

Compartment	Amount
Air	0.16%
Soil	79.3%
Water	20.4%
Sediment	0.14%

The predominant routes of loss were by reaction in the soil, water and air compartment (49.4, 25.4 and 14.0% respectively) and advection from the water compartment (10.3%). The estimated mean residence time for triethylene glycol dibenzoate in the model environment (persistence) was 503 hours.

Reliability: Estimated values based on accepted model.

References:

Triethylene Glycol Dibenzoate. Estimation of Environmental Fate Using the Mackay Level II Fugacity Model. Huntingdon Life Sciences. 2001.

VCL 273 - Benzoflex S-358. Determination of Physico-Chemical Properties. Huntingdon Life Sciences. 1998.

VCL366 – Triethylene Glycol Dibenzoate. Estimation of Photodegradation Using the Atmospheric Oxidation Program (AOPWIN). Huntingdon Life Sciences. 2001.

VCL 297 – Triethylene Glycol Dibenzoate. Preliminary Assessment of its Degradation in Laboratory and Natural Waters. Huntingdon Life Sciences. 1999.

Technical Guidance Document (TGD) in Support of Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances and Commission regulation (EC) No. 1488/94 on Risk Assessment for Existing Substances Part II, pp. 282-285.

Mackay, D., DiGuardo, A.., Paterson, S. and Cowan C. (1996) Evaluating the Environmental Fate of a Variety of Types of Chemicals Using the EQC Model. Environ Toxicol Chem, **15**, p. 1627.

BIODEGRADATION

Test Substance: See boiling point for purity

Test Method: OECD 301B, EEC Directive 92/69/EEC, Procedure C. 4-C

GLP: Yes

Test Type: Aerobic

Year Performed: 1997

Laboratory: Huntingdon Life Sciences

Inocculum: Activated sludge from sewage treatment works

Concentration: 10 mgC/l

Temperature of Incubation: 19.2 – 24.0°C

Duration: 29 days

Positive Control: Sodium benzoate plus inocculum mineral salts medium

Control and Blank: Mineral salts and medium plus inocculum

Results: 16% of TCO_2 @ 2days
62% of TCO_2 @ 7 days
92% of TCO_2 @ 28 days
Final mean level of biodegradation including residual CO_2 released from the medium after acidification was also 92%.

Readily biodegradable

Data Quality: 1, Reliable without restrictions

References: Benzoflex S-358 - Assessment of ready biodegradability - Modified Sturm Test. Huntingdon Life Sciences. 1998.

ACUTE TOXICITY TO FISH

Test Substance: Benzoflex S-358 is white solid.

NAME	CAS #	%
Triethylene Glycol Dibenzoate	120-56-9	96.9
Triethylene Glycol Monobenzoate	25022-51-7	1.28
Ethylene Glycol Monobenzoate	94-33-7	0.19
Diethylene Glycol Dibenzoate	120-55-8	0.16
Diethylene Glycol Monobenzoate	20587-61-5	0.12
Ethylene Glycol Dibenzoate	94-49-5	0.11
Unidentified Component #1	Unknown	0.15
Unidentified Component #2	Unknown	0.17

Test Method: OECD 203

Test Type: Acute Toxicity to Fish

GLP: Yes

Year Performed: 1997

Species/Strain: Fathead minnow (*Pimephales promelas*)

Analytical Monitoring: Every 24 hours

Exposure Period: 96 hours

Test Details: Semi-static conditions

Statistical Methods: LL_{50} values (median initial loading values) and 95% confidence limits were calculated using the Thompson and Weil model (Thompson and Weil, 1952).

Thompson, W.R. & Weil, C.S., 1952, Biometrics 8:51-54.

Test Condition Remarks:

Fish Size and Age: Juvenile *Pimephales promelas* mean standard length was 2.0 cm for Replicate 1 and 1.9 cm for Replicate 2. The mean weight was 0.10 g for Replicate 1 and 0.09 g for Replicate 2.

Test Conditions: As specified in OECD 203

Diluent Water Source and Chemistry:
Laboratory tap water filtered, dechlorinated and softened by passage through an Elga® water purification system. Chlorine levels ranged from 0.01 to 0.10 mg/L throughout the 7 days of the acclimatization period and the exposure period and the hardness level, calculated from daily measurements during the same period was between 73 and 76 mg CaCO3/L.

Stock and Test Solutions:
The method of preparation was selected following advice given in ECETOC 1996, Monograph No. 26. The test material is a complex mixture of components with poor but variable water solubility. In such cases it is considered that the water accommodated fraction is the most appropriate exposure medium.

Appropriate weights of the test substance, 20, 64, 200, 640 and 2000 mg, were dispersed into 20 liters of diluent water, in duplicate, contained in a glass reagent bottle. Each preparation was then stirred for at least 18 hours using a Stuart Scientific magnetic stirrer with a 10 cm magnetic follower to give water accommodated fractions with initial loading rates of 1.0, 3.2, 10, 32 and 100 mg/l. After stirring, the solutions were allowed to settle for 1 hour prior to removing the water accommodated fraction by peristaltic pump. Approximately 18 liters of solution were removed from a mid-water position and pooled to give the exposure solution.

Vessels and Lighting: Five test concentrations plus one control were prepared, each in duplicate. Ten fish were added to each vessel. Fish were placed at random in glass aquaria containing prepared test medium or diluent water, as appropriate. The test chambers were glass aquariums (25X46X25 cm) containing approximately 18 liters of medium to a depth

of 18 cm. Supplementary aeration was provided via narrow bore glass tubes. A photoperiod of 16 hours light: 8 hours dark was maintained and daily records of temperature, pH and dissolved oxygen were kept for each control and test vessel. The fish were not fed during the 96-hour exposure period.

Fish per Vessel: 10 fish in each vessel

Dose Selection: Nominal: 1.0, 3.2, 10, 32 and 100 mg/L.

Renewal and Exposure: Fish were exposed to the test or control conditions for a period of 96 hours with daily batchwise renewal of the test medium to ensure the maintenance of satisfactory environmental conditions and near optimal exposure levels.

Temperature range: Treatment and controls groups were maintained at 19°C throughout the exposure period.

Analytical Results:

Occasion	Nominal Concentration (mg/L)	Measured Concentration Triethylene Glycol Dibenzoate (mg/L)		
		Tank 1	Tank 2	Mean
0 Hours (Fresh)	Control	ND		
	1.0	0.6662		
	3.2	2.166		
	10	6.589		
	32	14.98		
	100	18.70		
24 Hours (Expired)	Control	ND	ND	ND
	1.0	ND	ND	ND
	3.2	ND	ND	ND
	10	ND	ND	ND
	32	ND	ND	ND
	100	ND	ND	ND
24 Hours (Fresh)	Control	ND		
	1.0	0.6470		
	3.2	2.275		
	10	4.465		
	32	14.78		
	100	14.96		
48 Hours (Expired)	Control	ND	ND	ND
	1.0	ND	ND	ND
	3.2	ND	ND	ND
	10	ND	ND	ND
	32	ND	ND	ND
	100	ND	ND	ND
48 Hours (Fresh)	Control	ND		
	1.0	0.6027		
	3.2	2.023		
	10	6.220		
	32	14.59		

	100	7.594		
72 Hours (Expired)	Control	ND	ND	ND
	1.0	ND	ND	ND
	3.2	ND	ND	ND
	10	ND	ND	ND
	32	ND	ND	ND
	100	ND	ND	ND
72 Hours (Fresh)	Control	ND		
	1.0	0.6401		
	3.2	1.844		
	10	5.536		
	32	2.108		
	100	4.331		
96 Hours (Expired)	Control	ND	ND	ND
	1.0	ND	ND	ND
	3.2	ND	ND	ND
	10	ND	ND	ND
	32	ND	ND	ND
	100	ND	ND	ND

ND – None detected (limit of detection: 0.0005 mg/L)

Results:

Time (hours)	**LL_{50} (mg/L)**	**95% confidence limits (mg/L)**
3-96	> 100	----

Highest loading rate resulting in 0% mortality: 10 mg/L
Lowest loading rate resulting in 100% mortality: > 100 mg/L
"No-observed effect loading rate": 10 mg/L

The measured concentration of triethylene glycol dibenzoate in fresh samples was between 11 and 65% of the total loading rate. The measured amount in exposure medium was greater proportionally as initial loading rate decreased. In the expired samples, triethylene glycol dibenzoate had degraded to levels below the limit of detection.

Environmental parameters (pH, T^0C, and mgO_2) remained within acceptable limits throughout the duration of the study.

Conclusions: The 96-hour LL_{50} value for Benzoflex S-358 with fathead minnow was > 100 mg/L. The no-observed effect concentration for Benzoflex S-358 with fathead minnow was 10 mg/l.

Data Quality: 1, Reliable without restrictions

References: Triethylene Glycol Dibenzoate. Acute Toxicity for Fathead Minnow (*Pimephales promelas*). Huntingdon Life Sciences. 2001.

ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Test Substance: See acute toxicity to fish for purity

Test Method: OECD 202 Part 1

Test Type: *Daphnia* Acute Immobilization Test

GLP: Yes

Year Performed: 1997

Species/Strain: *Daphnia magna*

Analytical Monitoring: Determination of test material concentration at 0 and 48 hours

Exposure Period: 48 hours

Test Details: Static without renewal

Statistical Methods: EL_{50} and 95% confidence limits were calculated using the Thompson and Weil model (Thompson WR and Weil CS, 1952, Biometrics 8: 51-54).

Test Condition Remarks:

Age at Initiation: Less than 24 hours

Test Conditions: As specified in OECD 202 following advice given in ECETOC 1996 Monograph No. 26.

Solvent: Reconstituted medium Elendt M4.

Vessel: 250 ml capacity glass jars containing 200 ml of prepared test medium, to give a loading of 40 ml of test solution / organism. The jars were loosely covered to minimize evaporative losses.

Daphnids per Vessel: 5

Dose Selection: Nominal initial loading rate: 4.6, 10, 22, 46 and 100 mg/L.

Temperature range: 20 +_1 °C

Solution pH: 7.6

Dissolved Oxygen: 7.4 – 8.3

Analytical Results:

Occasion	Nominal Initial Loading(mg/L)	% of Loading Monobenzoate (mg/L)	% of Loading Dibenzoate (mg/L)
0 Hours (Fresh)	Control	ND	ND
	4.6	15	55
	10	6	26
	22	7	37
	46	7	45
	100	4	28
48 Hours (expired)	Control	ND	ND
	4.6	9	0
	10	0	0
	22	23	7
	46	22	29
	100	11	20

ND = None detected (Limit of detection = 0.0003 mg/l)

Results:

Time (hours)	**EL_{50} (mg/L)**	**95% confidence limits (mg/L)**
24	< 100	-----
48	26	20-35

Highest initial loading rate resulting in 0% mortality: 4.6 mg/L
Lowest initial loading rate resulting in 100% mortality: > 100 mg/L
No-observed effect loading rate: 4.6 mg/L*

*10% immobilization was observed in the 4.6 mg/L test group. This level of immobilization is not considered significant, therefore the "no-observed effect loading rate" is 4.6 mg/L.

Nominal Initial Loading Rate (mg/L)	Cumulative immobilized *Daphnia magna* (initial population 5/replicate) 24 hours						Cumulative immobilized *Daphnia magna* (initial population 5/replicate) 48 hours					
	R1	R2	R3	R4	Total	%	R1	R2	R3	R4	Total	%
Control	0	0	0	0	0	0	1	0	1	0	2	10
4.6	0	0	0	0	0	0	0	0	2	0	2	10
10	0	0	0	0	0	0	2	0	0	1	3	15
22	0	0	0	0	0	0	1	2	1	4	8	40
46	0	0	0	0	0	0	4	5	5	5	19	95
100	0	0	0	0	0	0	4	5	5	4	18	90

R1 – Replicate 1
R2 – Replicate 2
R3 – Replicate 3
R4 – Replicate 4

Conclusions: The 48-hour EL_{50} was determined to be 26 mg/l. The No Observed Effect Concentration was 4.6 mg/l.

Data Quality: 1, Reliable without restrictions

References: Triethylene Glycol Dibenzoate. Acute Toxicity to *Daphnia magna.* Huntingdon Life Sciences. 2001.

ACUTE TOXICITY TO AQUATIC PLANTS

Test Substance: See acute toxicity to fish for purity

Test Method: OECD 201

Test Type: Algae Growth Inhibition Test

GLP: Yes

Date Performed: 1997

Species: *Selenastrum capricornutum,* Strain number CCAP 278/4

Element basis: Area under the curve (72 hours, 96 hours), growth rate (0-72 hours, 0-96 hours)

Exposure period: 96 hours

Test organisms: Sterile nutrient medium was inoculated from a master culture and cultured under continuous illumination (~7000 lux) in an orbital incubator at 22°C, to give an algal suspension in log phase growth, characterized by a cell density of 6.75 $x10^6$ cells/ml.

Test Conditions:

Test Temperature Range: 24 ± 1°C

Exposure Vessel Type: 250 ml conical flask each containing 100 ml of test or control culture were loosely stoppered and placed without conscious bias, in a Gallenkamp Illuminated Orbital Incubator.

Light levels and quality during exposure:
The cultures were incubated, without medium renewal for 96 hours under continuous illumination of approximately 7000 lux provided by 7X30 W "universal white" 1 meter fluorescent tubes.

Test Design:

Number of replicates: Seven test concentrations were prepared plus one untreated control, each in triplicate

Nominal Initial Loading Rates: 1.0, 2.2, 4.6, 10, 22, 46 and 100 mg/l

Analytical Results:

Occasion	Nominal Loading Rate (mg/L)	Measured Concentration	
		Monobenzoate (mg/l)	Dibenzoate (mg/l)
0 Hours (Fresh)	Control	ND	ND
	1.0	0.005861	0.3634
	2.2	0.024115	1.079
	4.6	0.05131	2.148
	10	0.1735	4.389
	22	0.3044	7.010
	46	0.4411	8.850
	100	0.5842	13.11
	10 no algae	0.1727	4.495
96 Hours (Expired)	Control	ND	ND
	1.0	ND	ND
	2.2	0.04458	ND
	4.6	ND	ND
	10	ND	ND
	22	ND	ND
	46	ND	ND
	100	ND	ND
	10 no algae	ND	ND

ND None detected (LOD: 0.003 mg/l for both the mono and dibenzoate)

Results:

	NOEL	EL_{10}	EL_{50}	EL_{90}
Area Under curve (72 hours)	10	9.9	33	>100
Growth Rate (0-72 hours)	22	15	>100	>100
Area Under Curve (96 hours)	10	9.3	33	>100
Growth Rate (0-96 hours)	22	24	>100	>100

*A No Observed Effect Loading Rate, 96-hour NOEL was 10 mg/L.

Mean cell density of control at 0 hour 1.11×10^4 cells/ml
Mean cell density of control at 96 hour 5.32×10^6 cells/ml

Chemical analyses of the two main components in fresh and expired water samples were carried out at 0 hours (fresh) and 96 hours (expired). The measured concentration of triethylene glycol dibenzoate in fresh samples was between 13 and 49% of the total loading rate. For triethylene glycol monobenzoate, the mean measured concentration in fresh samples was 2% of the total loading rate. Expired mean measured concentrations of both the mono and dibenzoate were generally below the limit of detection.

Conclusions: Benzoflex S-358 inhibited the growth of *Selenastrum capricornutum* at loading rates tested in excess of 10 mg/L under the conditions of this test.

The EL_{50} (Area under the curve) after 72 and 96 hours was 33 mg/L and the EL_{50} (Growth rate) from 0-72 and 0-96 hours was >100 mg/L.

Data Quality: 1, Reliable without restrictions

References: Benzoflex S-358. Algal Growth Inhibition. Huntingdon Life Sciences. 2001.

ACUTE TOXICTY

ORAL

Test Substance: See boiling point for purity

Study Type: Acute Oral

Test Method: OECD 401

GLP: Yes

Year Performed: 1997

Laboratory: Huntingdon Life Sciences

Species/Strain: Rat, Sprague-Dawley

Sex: Male and Female

Results: Acute Oral LD_{50} and 95% confidence limits
Male: 4843 (4198-5588) mg/kg body weight
Female: 3535 (2892-4322) mg/kg body weight
Combined: 4190 (3504-5072) mg/kg body weight

Results:

Number of Deaths at Each Dose Level:

3200 mg/kg:	1 female
5000 mg/kg:	3 males and 5 females

Time of Death of Each Animal:

Day 2	
5000 mg/kg:	1 male
5000 mg/kg:	3 females
Day 3	
5000 mg/kg:	2 males
5000 mg/kg:	2 females
Day 5	
3200 mg/kg	1 female

Description of Clinical Effects:

Piloerection was observed in all rats within 8 minutes of dosing. This sign persisted and was accompanied in rats later during the study by:

Hunched posture, waddling/unsteady gait, lethargy and ungroomed appearance in all rats at all dosages;

Partially closed eyelids in two females at 3200 mg/kg, in all rats at 5000 mg/kg;

Pallid extremities in all females at 2000 mg/kg and all rats at 3200 and 5000 mg/kg;

Increased salivation in one male at 2000 mg/kg, four females at 3200 mg/kg, all males and four females at 5000 mg/kg

Walking on toes in all rats at 2000 and 5000 mg/kg and all males and four females at 3200 mg/kg;

Respiratory distress (characterized by increased or decreased gasping or noisy respiration) in all rats at 2000 mg/kg, all males and four females at 3200 mg/kg and three males and all females at 5000 mg/kg

Clonic convulsions in four males at 5000 mg/kg

Increased lacrimation in one female at 2000 mg/kg and all males and two females at 5000 mg/kg;

Cold body surfaces in all males at 2000 mg/kg and in all rats at 3200 and 5000 mg/kg;

Prostration in one female at 3200 mg/kg and four males and two females at 5000 mg/kg

Stained muzzle and/or urogenital area in two females at 2000 mg/kg, in all rats at 3200 mg/kg and in four males and two females at 5000 mg/kg

Sensitivity to handling in one male and all females at 2000 mg/kg, all rats at 3200 mg/kg and four males and two females at 5000 mg/kg

Hyperactivity in one male at 500 mg/kg

Thin appearance in one female at 3200 mg/kg and one male at 5000 mg/kg

Straub tail in two males at 5000 mg/kg

Body tremors in one male and one female at 2000 mg/kg, four females at 3200 mg/kg and all males and two females at 5000 mg/kg;

Faecal disturbances (characterized by soft to liquid or lack of faeces) in all females at 2000 mg/kg and in two males at 5000 mg/kg;

Recovery of surviving rats was complete, with the exception of piloerection and unsteadiness in males at 5000 mg/kg, by either Day 10 (3200 mg/kg), Day 11 (2000 mg/kg), or Day 13 (males 5000 mg/kg).

Necropsy findings:

Females at 3200 mg/kg

Congestion (characterized by prominent blood vessels) in brain and gaseous distension in the stomach and along the alimentary tract.

Males and Females at 5000 mg/kg

Congestion (characterized by dark appearance/prominent blood vessels/inflammation) in brain, heart, lungs, liver, spleen and kidneys. Congestion or pallor with fluid contents, gaseous distension and black patches were also seen in the stomach and along the alimentary tract.

Data Quality: 1, Reliable without restrictions

References: Benzoflex S-358, Acute Oral Toxicity to the Rat, Huntingdon Life Sciences. 1998.

DERMAL

Test Substance: See boiling point for purity

Study Type: Acute Dermal Toxicity

Test Method: OECD 402

GLP: Yes

Year Performed: 1997

Laboratory: Huntingdon Life Sciences

Species/Strain: Rat, Sprague-Dawley

Sex: Male and Female

Results: Acute Dermal LD_{50} >2000 mg/kg

Number of Deaths at Each Dose Level: No deaths

Description of Clinical Effects: No signs of systemic reaction to treatment were observed in any animal throughout the observation period.

Data Quality: 1, Reliable without restrictions

References: Benzoflex S-358 Acute Dermal LD_{50}. Huntingdon Life Sciences. 1998.

REPEATED DOSE TOXICITY

Test Substance: See boiling point for purity

Vehicle: Administered by dietary admixture

Study type: Subchronic Oral Toxicity - Rat

Method: OECD 408

GLP: Yes

Year Performed: 1997 and 1998

Laboratory:	Huntingdon Life Sciences
Species/Strain:	Rat, Crl: (IGS) CD® BR
Age at Study Initiation:	~ 7-8 weeks
Route of Administration:	Dietary
Frequency of Treatment:	Continuous in diet
Duration of Test:	13 weeks with subsequent 4-week recovery for selected animals
Dose/Concentration Levels:	Control, 400, 1000, 1600 and 2200 mg/kg/day
Post-Exposure Observation:	10 male and 10 female rats at the control and 2200 mg/kg/day level served as a recovery group for a subsequent 4 weeks.

Clinical Observations Performed: Individual animals were observed and palpated at least once daily for any signs of behavioral changes, reaction to treatment or ill health. A detailed clinical observation was performed daily for the duration of the study. The weight of each rat was recorded at the time of allocation of animals to groups, on the day of commencement of treatment and once a week thereafter, including the day of death. The quantity of food consumed by each cage of rats was recorded weekly. Food conversion ratios were calculated, where possible, from the weekly bodyweight and food consumption data as weight of food consumed per unit gain in bodyweight. At weekly intervals, the group mean achieved intake of test substance (mg/kg/day) was calculated from the group mean bodyweight and food consumption and the dietary inclusion levels of the test material. Daily monitoring by visual appraisal of the water bottles was maintained throughout the study. The eyes of all rats were examined using a Keeler indirect opthalmoscope before dosing commenced. During Week 13, the eyes of all animals in Groups 1 and 5 were examined. As there was no effect of treatment, further investigations were not performed.

Results:

NOAEL: 1000 mg/kg/day is considered to represent the No Observable Effect Level of Benzoflex S-358 in rats by oral administration for 13-weeks.

Actual Dose Received: Overall Achieved Intakes of Benzoflex S-358

Group 2 Males: 395 mg/kg/day
Group 3 Males: 987 mg/kg/day
Group 4 Males: 1576 mg/kg/day
Group 5 Males: 2185 mg/kg/day

Group 2 Females: 398 mg/kg/day
Group 3 Females: 1015 mg/kg/day
Group 4 Females: 1596 mg/kg/day
Group 5 Females: 2189 mg/kg/day

Toxic Response/Dose Level:

Mortality: There was one unscheduled death that was sacrificed due to poor condition in Week 12. Microscopic pathology revealed the factors contributory to death were lymphoblastic/lymphocytic lymphoma. This death was not associated with treatment.

Clinical Signs: During the treatment period, hair loss was apparent in 6 of 20 males and 3 of 20 females at 2200 mg/kg/day in comparison with 0 of 20 males and 4 of 20 female control animals. During the recovery period, hair loss was apparent in 2 of 9 male and 6 of 10 females at 2200 mg/kg/day in comparison with 1 of 10 male and 3 of 10 female control animals. The finding was first noted from Week 15 for the male animals and from Week 4 for 1 female, Week 12 for 2 of the female animals and from Week 15 for the remaining females.

Bodyweight: Bodyweights were decreased in both sexes at the two highest dose levels. This was more apparent in males than females. At 1000 mg/kg/day and lower dose levels, no biologically significant bodyweight decrease was observed. This was determined by final total bodyweights and by bodyweight gain. While females showed a statistically significant lower bodyweight gain at Week 13 given 1000 mg/kg/day and above, there was no dose response exhibited nor was there any dose response observed for final total bodyweights. Moreover, no female dose level exceeded an 8% decrease for final bodyweights. Therefore this indicates that a 1000 mg/kg/day dose is the minimal no-effect-level for bodyweight changes.

Food Consumption: Food consumption for the first week of treatment for males at 2200 mg/kg/day was slightly lower than controls, the value attaining statistical significance. During the same period females at 400, 1600 and 2200 mg/kg/day also showed a lower food intake when compared with controls but with no effect at 1000 mg/kg/day and no dose relationship apparent. Food consumption for both males and females during Weeks 2 to 13 was generally comparable for all treated groups. During Weeks 1 to 13, a slightly lower food consumption was noted for males at 2200 mg/kg/day in comparison with controls, this value attaining statistical significance. During the same period both sexes at 400 mg/kg/day demonstrated a decreased food consumption though these values did not attain statistical significance, all other treated groups were generally comparable with control values and consequently no relationship to treatment is suspected. During the recovery period the food consumption for both males and females at 2200 mg/kg/day was generally comparable with their respective control groups.

Water Consumption: Analysis of the Week 13 water consumption revealed that the intakes for each treated group were comparable with their respective control groups that there was no effect of treatment. As a result, water consumption was not assessed during the recovery period.

Ophthalmic Investigation: Week 13 ophthalmic investigation revealed no changes that were attributable to treatment. All findings were characteristic of the age and strain of animals employed. Ophthalmoscopy was therefore not performed during the recovery period.

Hematology: When measured at Week 5 in males only at 1600 and 2200 mg/kg/day dose groups, red blood cell indicators suggested an increase in RBC's. However, at Week 13 the same parameters were not consistent in males. Females did not show the same trend with the same parameters at either Week 5 or 13. Similarly, males showed a non-cellular specific decrease in white blood cells at the two highest dose levels; but the females showed no indications of a decrease in WBC's at any dose level. An integrated interpretation might suggest a decrease in RBC parameters with the excess haemosiderin in the spleen, suggesting a breakdown of circulating RBC's. However, if this were the case the reticulocyte count would be elevated indicating synthesis of new RBC's. This is not noted in either sex at any dose level. While the increased RBC's in males may or may not be treatment related, the toxicological significance remains obscure. Moreover, no biologically significant alterations were noted for any haematological parameters at the 1000 mg/kg/day level or below.

Biochemistry: Analysis of the Week 5, Week 13, and Week 17 biochemistry investigation revealed a number of statistically significant differences between the control and treated groups but all of these were slight with no clear shifts in the range of the individual values or consistency between the weeks and sexes.
The parameters showing the clearest shift in range of individual values from controls were:

In Week 5, very slight increases in ALT and AST were noted in both sexes at 2200 mg/kg/day plus females at 1600 mg/kg/day was noted. In Week 13 a similar finding was still apparent for the males only at 2200 mg/kg/day and was associated with periportal hepatocyte hypertrophy seen in the majority of males at 2200 mg/kg/day and an occasional female at 2200 mg/kg/day. This difference was not apparent for the Week 17 investigation and there was no residual hepatic pathology.
In Weeks 5, 13 and 17, a slight decrease in cholesterol in females at 2200 mg/kg/day was noted. As this finding was not apparent for males at this dose level, it is considered not to be of toxicological importance.
In Weeks 5 and 13, a slight increase in triglycerides was noted in females at 2200 mg/kg/day. This change was not apparent at the Week 17 investigation and was not seen in male animals at this dose level. It is considered not to be of toxicological importance.

Gross pathology: The macroscopic examination at the terminal *post mortem* revealed alopecia in 4 of 10 animals receiving 2200 mg/kg/day in comparison with 0 of 10 controls, while at the recovery *post mortem* alopecia was seen in 2 of 9 male and 4 of 10 female animals receiving 2200 mg/kg/day, these findings were related to the in life clinical observation of hair loss. The incidence and distribution of all other findings was considered to fall within the expected background range of macroscopic changes and was not related to treatment.

Organ Weight Changes: All organ weights were generally comparable with the concurrent control groups and there were no findings considered of toxicological importance. Statistical analyses of the organ weights revealed several values attaining statistical significance however, the differences were generally slight and inconsistent between the sexes. The only change showing a degree of consistency was a slight increase in lung weight in both sexes receiving 2200 mg/kg/day and males receiving 1600 mg/kg/day, which was not apparent during the recovery kill.

Histopathology: Treatment Period - *Liver:* Periportal hepatocyte hypertrophy was seen in the majority of males and an occasional female rat receiving 2200 mg/kg/day. *Spleen:* Haemosiderosis is a normal physiological response to red cell turnover in rats and is most frequently present to a minimal degree in control rats. However, an increased incidence and degree of haemosiderosis was seen in male and female rats receiving 2200 mg/kg/day. This was considered to be a treatment-related exacerbation of this physiological change.
Recovery Period – *Spleen:* The degree of haemosiderosis was still increased in male and female rats receiving 2200 mg/kg/day, although there was evidence of recovery. A slight increased incidence of minimal extramedullary haemopoiesis was seen in male and female rats receiving 2200 mg/kg/day after the 4-week recovery period. *Liver*: No treatment-related changes were detected in rats receiving 2200 mg/kg/day after the 4-week recovery period. The periportal hepatocyte hypertrophy seen in the liver of rats in this study at the end of the treatment period were therefore considered to be reversible.

Statistical Analysis: All statistical analyses were carried out separately for males and females. Data relating to food and water consumption were analyzed on a cage basis. For all other parameters, the analyses were carried out using the individual animal as the basic experimental unit. Food consumption data were analyzed using cumulative totals and water consumption data were analyzed as the total recorded intake over a selected time period, expressed on a weekly basis. Bodyweight data were analyzed using weight gains.

Discussion and Conclusion: Lower bodyweight gains were apparent for both sexes receiving 1600 or 2200 mg/kg/day. A slight reduction in food intake was noted for high level males. Dietary administration of the test substance at 2200 mg/kg/day correlated with pathological changes in the liver and spleen. Changes in or associated with the liver included an increase in plasma enzyme activities and periportal hepatocyte hypertrophy. Changes in the spleen increased in incidence and degree of haemosiderosis.

During a 4 week recovery period bodyweight gain for animals previously receiving 2200 mg/kg/day was superior to that of the controls such that the intergroup deficit was almost offset. Following recovery, liver enzyme activity was normal and there was no residual pathology. The incidence and degree of haemosiderosis was reduced but recovery was not complete.

In conclusion, 1000 mg/kg/day was a No Observed Effect Level. Dosages of 1600 and 2200 mg/kg/day were tolerated over a 13-week period but induced some or all of the changes in blood parameters, minor treatment-related pathology and adverse effects on bodyweight gain and food consumption. When selected animals previously receiving 2200 mg/kg/day were maintained off-dose for 4-weeks, all treatment related changes showed complete or tended towards recovery.

Data Quality: 1, Reliable without restrictions

References: Benzoflex S-358. Toxicity to Rats by Dietary Administration for 13 weeks with Subsequent 4-Week Recovery Period for Selected Animals. Huntingdon Life Sciences. 1999.

DEVELOPMENTAL TOXICITY

Test Substance: Benzoflex S-358 is white solid.

NAME	CAS #	%
Triethylene Glycol Dibenzoate	120-56-9	99.249%
Triethylene Glycol Monobenzoate	25022-51-7	0.551%
Unknown Component #1	Unknown	0.155%

Method: US EPA 870.3700 Harmonized Guideline, with the following exception:

The guideline states that "Evaluation of the dams during cesarean section and subsequent fetal analyses should be conducted without knowledge of treatment group in order to minimize bias". Evaluation was made with knowledge of the treatment group, as procedures are already in place to minimize bias during these portions of the study. These procedures include routine reviews of necropsy technicians evaluation skills and scientific peer review of at least 25% of the raw data of the fetal analyses, including examination of serial sections for visceral anomalies and examination of fetal skeletons.

GLP: Yes

Year Performed: 1998

Laboratory: Huntingdon Life Sciences

Species/Strain: Rat, Sprague-Dawley CD

Route of Administration: Oral (gavage)

Dosages: 0, 250, 500 and 1000 mg/kg/day

Number and Sex: 22 Pregnant Females / Group

Exposure period: Days 6 – 19 of gestation inclusively

Frequency of Treatment: Daily

Control Group: Corn Oil Vehicle

Duration of Test: Necropsy at Gestation Day 20

Statistical Evaluation: Statistical tests, employing analysis of variance followed by an inter-group comparison with the Control, were performed on the following parameters: Bodyweight change, bodyweight change adjusted for gravid uterine weight, food consumption, litter data, litter weight, fetal weight and placental weight.

Dependant on the heterogeneity of variance between treatment groups, parametric tests (analysis of variance Snedecor and Cochran 1967) followed by Williams' test (Williams 1971/2) or nonparametric tests (Kruskal-Wallis, Hollander and Wolfe 1973) followed by Shirley's test (Shirley 1977) were used to analyze these data, as appropriate. Where 75% or more of the values for a given variable are the same, a Fisher exact test (Fisher 1950) was used.

For litter data (excluding fetal, litter and placental weights) and implantation loss, due to the preponderance of non-normal distributions, non-parametric tests are generally the most consistent and were routinely used.

All significant (i.e.$p<0.5$) inter-group differences from the Control are reported only where supported by a significant analysis of variance (i.e. $p<0.0.5$)

FISHER, R.A. (1950) Fisher's exact test 2x2 contingency table:*Statistical Methods for Research Workers*, para. 21.02 Oliver and Boyd, Edinburgh.

HOLLANDER, M and WOLFE, D.A. (1973) *Non-parametric statistical methods.* Publ. J. Wiley and Sons, New York. KRUSKAL-WALLIS and JONCKHEERE tests: 114-132.

SHIRLEY, E. (1977) A non-parametric equivalent of William's test for contrasting increasing dose levels of a treatment. *Biometrics*, 33: 386-389.

SNEDECOR, G.W. and COCHRAN, W.G. (1967) *Statistical methods.* 6th ed. The Iowa State University Press.

WILLIAMS, D.A. (1971/2) William's test for comparing the effect of increasing doses of substance with a zero dose. *Biometrics* 27: 103-117. *Biometrics*, 28: 519-531.

Remarks:

Age at Study Initiation: 10 to 11 weeks of age

Test Substance Preparation: Dosages were formulated in corn oil on a weekly basis. They were prepared by weighing as a solid, appropriate amounts of the test substance and then warming them to 60°C to liquefy them. The test substance was then added to an appropriate amount of corn oil, which was also warmed to 60°C and stirred for ten minutes maintaining a temperature of approximately 45°C to 50°C during preparation and sampling.

Clinical Observation (Maternal): All animals were observed at least twice daily throughout the study for any visible signs of reaction to treatment. Observations associated with dosing were also recorded during the treatment period according to the following schedule: 1) Pre-dosing, 2) On return of animal to home cage, 3) After dosing each group, 4) 1 to 2 hours after completion of dosing all groups, 5) As late as possible in the working day. Maternal bodyweight was measured

on Days 0,3, 6 to 17 inclusive and 20 after mating. Food consumption was recorded for the periods Days 0-2, 3-5, 6-8, 9-11, 12-14, 15-16 and 17-19 after mating.

Mating Procedure: Females were paired on a one-to-one basis with stock males of the same strain. Each morning following pairing, the trays beneath the cages were checked for ejected copulation plugs and a vaginal smear was prepared from each female and examined for presence of spermatozoa. The day on which a sperm positive vaginal smear or at least 3 copulation plugs were found was designated Day 0 of gestation.

Terminal Observations (Maternal): On Day 20 after mating, the females were weighed at necropsy and then killed by inhaled carbon dioxide for examination of their uterine contents. Each animal was first examined macroscopically for evidence of disease or adverse reaction to treatment and specimens of abnormal tissue were retained. The reproductive tract, complete with ovaries, was dissected out and the following recorded: 1) Gravid uterine weight – uterus with cervix, 2) Number of corpora lutea in each ovary (assessed prior to removal), 3) Number of implantation sites, 4) Number of resorption sites (classified as early or late), 5) Number and distribution of fetuses in the uterine horn.

Fetal Examination: Each fetus was weighed, sexed and examined for any external abnormalities. Individual placental weights and placental abnormalities were recorded. Fetuses were killed by chilling on a cool plate. The neck and thoracic and abdominal cavities of approximately half of each litter were dissected and examined. Fetal changes were recorded and the offspring eviscerated prior to fixation in Industrial Methylated Spirit. After fixation, fetuses were processed, stained with Alizarin Red and skeletal development assessed. The remaining fetuses in each litter were placed in Bouin's fixative, subjected to free hand serial sectioning and examined for visceral changes.

Results:

Maternal Toxicity

NOEL: 1000 mg/kg/day

Clinical Signs: The general condition of females at all dosages was satisfactory and there were no unscheduled deaths.

In treated groups, salivation after dosing was observed in all animals. This sign was first observed in one animal receiving 1000 mg/kg/day on the first day of treatment. The incidence increased on subsequent days with most animals affected from the fifth day of treatment onwards. The number of days on which individual animals were affected was greatest at 1000 mg/kg/day and showed an apparent dosage-relationship. The post-dosing salivation seen at the other dosages was at a lesser incidence. In all cases this sign was transient in nature and probably due to the palatability of the test substance.

During treatment the food intake of animals receiving 1000 mg/kg/day and to a lesser extent those receiving 500 mg/kg/day was slightly increased when compared with the Control values. Despite some statistical significance, these differences were not considered to reflect an adverse effect of treatment.

Litter Responses and Fetal Changes

Litter responses on Day 20 of gestation: All females in each group had live young at Day 20 of gestation. Pre-natal survival as indicated by the extent of pre- and post-implantation loss and the numbers of live fetuses was unaffected by treatment. The mean percentage of males per litter was comparable in all groups.

There were no apparent effects of treatment on mean placental weights but there was a reduction in fetal weights at 1000 mg/kg/day when compared with the Control values.

Although this effect was slight, it was statistically significant in both the male and female fetuses and was considered to be treatment related.

Fetal Changes: There were no apparent effects of treatment at 250 or 500 mg/kg/day.

At 1000 mg/kg/day, treatment was associated with an increase in the number of fetuses /litters with cervical ribs when compared with the concurrent controls, the other treated groups and background control data. Additionally, there were also a greater number of fetuses with incomplete ossification of the cranial centers, sacrocaudal vertebral arches and pelvic bones compared with Controls and background data. These are considered to be related to treatment with 1000 mg/kg/day.

There was an increased incidence of incomplete ossification of the 5th and/or 6th sternebrae in the Control and all treated groups. Since there was no marked increase in this finding above the concurrent control and there was no dose-response relationship, this finding was not considered to be an adverse effect of treatment.

The incidence of rudimentary or absent renal papilla and dilated ureter showed an apparent increase in animals treated. These increased incidences did not exhibit an obvious dosage-relationship and as such these were not considered to be an adverse effect of treatment.

Analytical Results:

Group: **Dosage:** (mg/kg/day) **Volume:** 5 ml/kg	1 0	2 250	3 500	4 1000
Adult Females				
Females with sperm	22	22	22	22
Pregnant Females	22	22	22	22
Evaluated Pregnant Females	22	22	22	22
Litters – group mean values				
Corpora lutea	16.2	16.6	17.3	16.0
Implantation	15.7	15.5	16.4	15.3
Resorptions	0.7	1.3	0.8	0.9
Live fetuses	15.0	14.3	15.6	14.5
Sex ratios of fetuses (%)				
Male	44.2	50.3	51.7	50.4
Weight of fetuses (g)				
Male	3.91	3.90	3.94	3.75
Female	3.72	3.70	3.70	3.55
Important Fetal Findings				
Number of fetuses (litters) with:				
Fetal malformations: None significant				
Fetal anomalities:				
Cervical ribs *	1(1)	1(1)	2(2)	5(3)
Incomplete ossification:				
Cranial centers	0	4(3)	4(4)	13(9)
Sacrocaudal vertebrae	3(2)	2(2)	4(3)	15(9)
Pelvic bones	0	0	3(2)	8(6)
* Not considered to be of long-term toxicological significance				

Conclusions:

The test substance administered orally to pregnant rats from Day 6 to 19 of pregnancy, at dosages of up to 1000 mg/kg/day, was well tolerated and there were no apparent signs of adverse general maternal toxicity. Post-dose salivation was observed at all dosages of but this was most probably due to palatability of the test substance.

At 250 and 500 mg/kg/day there were no apparent effects on pre-natal survival or fetal development.

At 1000 mg/kg/day, there was no effect of treatment on pre-natal survival but fetal weights were low compared with Controls (about 4% lower). There was also an increase in the incidence of fetuses with incomplete ossification of the cranial centers, sacrocaudal vertebral arches, pelvic bones and 5th and/or 6th sternebrae when compared with the Control group. The effects on ossification reflects the lower fetal weight recorded at this dosage; these findings are consistent with a slight retardation in fetal growth towards the end of gestation and may be related to the extended treatment period; delays in ossification of this nature are generally considered to be transient in nature, rather than representing permanent structural changes, and therefore judged to be of doubtful toxicological significance. The finding of an increased incidence of cervical ribs at 1000 mg/kg/day was considered to be of greater toxicological significance as it occurred at a dosage that produced no detectable signs of maternal toxicity. However, the presence of cervical ribs was only detected in a small number of fetuses (5 out of 156 examined at this dosage) and there were no accompanying changes in vertebral configuration.

In conclusion, it is considered that 1000 mg/kg/day is the no-effect-level for maternal toxicity. Apart from the occurrence of a small number of fetuses with cervical ribs at 1000 mg/kg/day, the no adverse effect level for pre-natal development was concluded to be 1000 mg/kg/day. The no-effect-level for all aspects of pre-natal development is concluded to be 500 mg/kg/day.

Reliability: 1, Reliable without restrictions

Reference: Benzoflex S-358. Study of Prenatal Development in the CD Rat by Oral Gavage Administration. Huntingdon Life Sciences. 2000.

REPRODUCTIVE TOXICITY

Test Substance: See developmental toxicity for purity

Method: USEPA OPPTS 870.3800 (1998)

Type: One Generation

GLP: Yes

Year Performed: 1999

Laboratory: Huntingdon Life Sciences

Species/Strain: Rat, Sprague-Dawley (CD-IGS)

Route of Administration: Dietary - continuous

Doses: 2500, 5000, 10000 and 20000 ppm

Sex: Male and Female

Animals / Dose: 10 male and 10 female

Control Group: Yes, basal diet without the test material

Frequency: Continuously in diet

Duration: Approximately 13 weeks

Premating Exposure Period for Male and Females: 15 days prior to pairing and continued uninterrupted until termination after weaning of the litters. Selected animals from the F1 generation then received the diets from about the time of weaning until termination following attainment of sexual maturation.

Statistical Method: No statistical analysis was performed because of the small sample size.

Remarks: This study was performed to assess the influence of Benzoflex S-358 in the diet on gonadal function, mating behavior and fertility in sexually mature male and female CD rats. Assessment of the survival and development to sexual maturity of the F1 generation and the tolerance of selected offspring to exposure at the same dietary concentrations as the parental animals were also determined. For this purpose, Benzoflex S-358 was administered orally, via the diet, at inclusion levels of 2500, 5000, 10000 and 20000 ppm to groups of 10 males and 10 females.

Mating Procedure: After the scheduled period of treatment, the Fo females were paired on a one-to-one basis with males from the same treatment group. Each morning following pairing, the trays beneath the cages were checked for ejected copulation plugs and a vaginal smear was prepared from each female and examined for the presence of spermatozoa. The day on which a sperm positive vaginal smear or at least three copulation plugs were found was designated Day 0 of gestation. Once mating had occurred, the males and females were separated and vaginal smearing discontinued.

Parameters Assessed During Fo and F1: All animals were inspected at least twice daily throughout the study and any visible signs of reaction to treatment were recorded, with details of type, severity, time of onset and duration. Animals killed for reasons of animal welfare were subjected to a thorough macroscopic examination of the visceral organs and specimens of abnormal tissues were retained. Males were weighed on the first day of treatment and weekly thereafter until termination. Females were weighed on the first day of treatment and then weekly until mating was detected. Subsequently the females were weighed on Days 0, 6, 13, and 20 after mating and on Days 1, 4, 7, 14 and 21 of lactation. F1 selected animals were weighed weekly from nominal 4 weeks of age until termination following sexual maturation (approximately 8 weeks of age). Food consumption was recorded weekly for the Fo animals until they were paired for mating. Food consumption for the females was recorded for the periods 0-5, 6-12 and 13-19 days after mating and days 1-3, 4-6 and 7-13 during lactation. Food consumption for the F1 selected animals was recorded weekly for nominal Weeks 4 to 5 and 5 to 6 of age – described as Weeks 4 or 5 in the results.

Estrous Cycles: For 10 days before pairing of the Fo animals, vaginal smears were taken daily from all females to establish the normality or otherwise of the estrous cylce. This was continued after pairing with the male until evidence of mating was observed.

Results:

Fo generation
The general condition of the Fo generation was satisfactory throughout the study. There were no unscheduled deaths amongst the males. Four females were killed, for reasons of animal welfare, before scheduled termination. These all occurred in early lactation and were linked with total littler loss (peri-natal mortality) or maternal neglect of the neonates. The litters from these females, where necessary, were also killed for reasons of animal welfare. The deaths were: one at 2500 ppm, one at 10000 ppm and two at 20000 ppm. The circumstances of these deaths did not suggest a dose or treatment-related effect.

Overall, there were no obvious adverse effects on overall parental bodyweights or food consumption and food conversion efficiency was considered to be similar to controls. The achieved dosage at all dietary concentrations for both sexes was considered to be satisfactory and exposure of Benzoflex S-358 at over 1500 mg/kg/day was achieved, in the top dose group, during the two weeks before pairing.

Estrous cycles, mating performance, fertility, gestation length, parturition and fecundity were similar in all groups. Necropsy of the parent animals revealed no macroscopic findings that were considered to be related to treatment. Organ weight assessment of the parent animals and histological examination (Groups 1 and 5) of their reproductive organs did not suggest any adverse effects on the reproductive organs of either sex.

Litter responses
The general condition of the F1 offspring showed no adverse effects of treatment with Benzoflex S-358. The number of uterine implantation sites, litter size, F1 offspring survival, sex ratio and F1 bodyweights showed no adverse responses as a result of exposure to the test material; either indirectly (*in utero* or through the milk) or directly as weaning occuured. Macroscopic examination of the F1 offspring not selected to form an F1 generation did not reveal any evidence of an adverse effect of treatment.

F1 generation
The general condition of the developing F1 animals reared to sexual maturity was satisfactory throughout. The overall development, bodyweights, food consumption and food conversion efficiency were considered to be similar to the control values. Achieved dosages were as expected, generally higher than that of their parents at the start of the study.

The timing of vaginal opening and balano-preputial separation (sexual maturation) were similar in all groups. Necropsy of the F1 animals revealed no findings that were considered to be related to treatment with Benzoflex S-358.

Analytical Results:

Group: Dietary Concentration (ppm):	1 0	2 2500	3 5000	4 10000	5 20000
Parental Animals					
Number females with normal estrous cycle	10	10	10	10	9
Number males/females paired 1:1	10	10	10	10	10
Number females with sperm	10	10	10	10	10
Pregnant females	10	10	10	10	10
Females with delivery	10	10	10	10	10
Important parental findings					
None considered to be related to treatment					
Important findings for F1 post-weaning progeny					
None considered to be related to treatment					
Litters					
Implantations, assessed at termination - mean	15.2	14.8	14.1	15.0	14.1
Live litters	10	10	10	10	10
Sex ratio Day 1 after birth (as %M) – mean	48.5	49.8	49.3	51.0	57.3
Surviving litters at Day 4 after birth	10	9	10	9	8
Number offspring Day 4 after birth (before culling) - mean	13.4	12.6	13.4	14.0	12.9
Number offspring Day 4 after birth (after culling) - mean	9.8	9.6	10	10.0	10.0
Number litters at Day 21 – weaning	10	9	10	9	8
Surviving litters at Day 21 - mean	9.8	9.4	9.9	10	10
Weight at birth (g) - mean					
Males	6.1	6.0	5.8	5.9	6.1
Females	5.6	5.7	5.5	5.5	6.0
Weight at weaning (g) - mean					
Males	49.1	45.2	47.2	47.4	45.0
Females	47.2	42.8	43.8	45.1	43.9

Conclusion:

Dietary administration of Benzoflex S-358 at concentrations of 2500, 5000, 10000, or 20000 ppm was well tolerated by the parental animals and their progeny. Satisfactory exposure to the test material was achieved throughout the study as judged by calculations of achieved intake and periodic dietary analysis. There were no obvious toxicological effects of treatment on the general condition of the parental animals or on their fertility and reproductive performance.

Litter parameters at birth of the F1 progeny, their survival and development to sexual maturity showed no apparent adverse effects of treatment with Benzoflex S-358.

There were no apparent abnormal findings at necropsy of the parental animals, the post-weaned unselected F1 offspring or the selected F1 animals. Organ weight assessment of the parent animals and histological examinations of reproductive organs (Control and 20000 ppm) did not suggest any adverse effects on these organs of either sex.

The evidence from this preliminary study suggested that administration of Benzoflex S-358, in the diet at levels up to and including 20000 ppm, was without apparent toxicity to the reproductive system of the adult rat and without impairment of growth and development of their progeny.

Additional Comments:

This preliminary study did not show any indication of adverse reproductive effects in rats given triethylene glycol dibenzoate (Benzoflex S-358). This preliminary study was conducted on 10 animals per group given up to 20,000 ppm in their diet. No observation of any specific parameters related to reproductive performance or any other toxicity was observed. Due to the lack of indications of any adverse effects, a second generation was not conducted. Nevertheless, if a standard two-generation reproduction study were conducted on this material, it would be highly unlikely that any specific adverse reproductive parameter would be observed. Two related materials, diethylene glycol dibenzoate and dipropylene glycol dibenzoate (Benzoflex 2-45 & Benzoflex 9-88, respectively) have been tested thoroughly in standard two-generation reproduction studies. Neither substance was observed to cause any specific adverse reproductive effects at doses up to 10,000 ppm given in the diet. These closely related substances support the judgment that Benzoflex S-358 also would not cause any specific adverse reproductive effects if tested in a standard two-generation study. Furthermore, studies on the latter materials showed no evidence of mutagenic, estrogenic, nor specific adverse developmental effects (teratogenic) when tested in and under the appropriate protocols for regulatory compliance.

The conclusion above, judging that no specific adverse effect would occur in a two-generation reproduction study with triethylene glycol dibenzoate (Benzoflex S-358) is based on considerable knowledge. The toxicological profile of this series of substances, ethylene glycol dibenzoates, is remarkably similar. The developmental (teratogenic effects), mutagenic, estrogenic, and very importantly the 13-week subchronic studies showed very similar effects for diethylene glycol dibenzoate and dipropylene glycol dibenzoate, (Benzoflex 2-45 & Benzoflex 9-88, respectively), as did those same studies conducted on Benzoflex S-358. Moreover, if a specific reproductive effect were to occur in the second generation of a two-generation study, the process by which generational changes occur would most likely be through mutagenicity. Since these substances are not mutagenic, the probability of a second generation effect, taken with the similarity of the bulk of previous data available on all of the ethylene glycol dibenzoates, strengthens this conclusion.

Reliability: 1, Reliable without restrictions

Reference: Benzoflex S-358. Preliminary Study of Effects on Reproductive Performance in CD Rats by Dietary Administration

AMES MUTAGENICITY

Test Substance: See boiling point for purity

Solvent: DMSO

Study Type: Bacterial reverse mutation assay

Test Method: OECD 471, 472

GLP:	Yes	
Year Performed:	1997	
Laboratory:	Huntingdon Life Sciences	
Species/Strain:	S. typhimurium:	TA 1535 his G46 rfa uvrB TA 1537 his C3076 rfa uvrB TA 98 his D3052 rfa uvrB pKM101 TA 100 his G46 rfa uvrB pKM101
	E. coli	CM891 WP2 trp uvrA pKM101
Concentrations:	5000, 1500, 500, 150, 50, 15, 5 μg/plate	
Metabolic Activation:	Sprague-Dawley rat liver	
Quantity of Activator:	0.5 ml	
Induction:	Stimulated by Aroclor 1254	

Criteria for Evaluating Results:

(a) If treatment with test substance produces an increase in reverent colony numbers of at least 2 times the concurrent solvent controls with some evidence of a positive dose relationship, in a specific bacterial strain reproduced with or without S9 mix, it is considered to show evidence of mutagenic activity in this test system. No statistical analysis is performed.

(b) If treatment with a test substance does not produce reproducible increases of at least 1.5 times the current solvent controls, at any dose level, with any bacterial strain, it is considered to show evidence of mutagenic activity in this test system. No statistical analysis is performed.

(c) If the results obtained fail to satisfy the criteria for a clear "positive" or "negative" response given in paragraphs (a) and (b), additional testing may be performed in order to resolve the issue of the substance's mutagenic activity in this test system. Modifications to the experimental method will usually be considered, such as the use of a narrower dose range and different levels of S9 mix. Should an increase in reverent colony numbers then be observed which satisfies paragraph (a) the substance is considered to show evidence of mutagenic activity in this test system. If no clear "positive" response can be obtained, the test data may be subjected to analysis to determine the statistical significance of any observed increases in reverent colony numbers. The statistical procedures used will be those described by Mahon *et al* (1989) and will usually be analysis of variance followed by Dunnett's test.

Mahon, G.A.T., Green, M.H.L., Middleton, B., Mitchell, I de G., Robinson, W.D. and Tweats, D.J. (1989). Analysis of data from microbial colony assay in: Kirkland, D.J. (ed.) *UKEMS Subcommittee on Guidelines for Mutagenicity Testing. Report Part III. Statistical Evaluation of Mutagenicity Data.* P.26. Cambridge University Press. Cambridge.

Positive/Negative Controls: Positive without S9:
N-Ethyl-N'-nitro-N-nitrosoguanidine in DMSO @ 5μg/plate for TA 1535, 3μg/plate for TA 100 and 2μg/plate for CM891

9-Aminoacridine in DMSO @80 μg/plate for TA 1537

	2-Nitrofluorene in DMSO @ 1μg/plate for TA 98 Positive with S9 2-Aminoanthracene in DMSO @ 2μg/plate for TA 1535 and 10μg/plate for CM891 Benzo[a] pyrene in DMSO @ 5μg/plate TA 1537, TA 98 and TA 100 Negative DMSO
Repeat Test:	The second test includes a preincubation stage. The first test is only the Standard Plate Incorporation.
Results:	
Cytotoxic Concentrations:	No toxicity with or without Metabolic Activation.
Precipitation Concentration:	5 mg/plate – cloudy solution 1.5 mg/plate – cloudy solution 0.5 mg/plate – slightly cloudy solution 0.15 mg/plate – no observable cloudiness A slight reduction in cloudiness occurred during the incubation period.
Genotoxic Effects:	No genotoxic effects observed with or without metabolic activation.
Conclusion:	No evidence of mutagenic activity in this bacterial system.
Data Quality:	1, Reliable without restrictions
References:	Benzoflex S-358. Bacterial Mutation Assay (*S.typhimurium* and *E. coli*). Huntingdon Life Sciences. 1998.

MAMMALIAN CELL GENE MUTATION

Test Substance:	See boiling point for purity
Solvent:	DMSO
Study Type:	*In vitro* mammalian cell gene mutation tests.
Test Method:	OECD 476
GLP:	Yes
Year Performed:	1997
Laboratory:	Huntingdon Life Sciences

Species/Strain: Mouse lymphoma L5178Y

Concentrations: 0, 100, 150, 200, 300, 325, 400 μg/ml

Metabolic Activation: Sprague-Dawley rat liver

Quantity of Activator: 4 ml

Induction: Stimulated by Aroclor 1254

Criteria for Evaluating Results:

Criteria for a positive response:

An increase of at least 100 in the mutant frequency in treated cultures relative to the concurrent control.
The demonstration of a statistically significant increase in mutant frequency following treatment with the test substance.
Evidence of a dose relationship over at least two consecutive dose levels, in any increases in mutation frequency.
Demonstration of reprodicibility in any increase in mutant frequency.
An increase in absolute colony numbers in the treated cultures.
The RTG of cultures showing an increase in mutant frequency should not be less than 10%.

Positive/Negative Controls: Positive without S9:
Methylmethane sulphonate in DMSO – 10ug/ml
Positive with S9:
20-methylcholanthrene in DMSO – 2.5 ug/ml

Results:

Genotoxic Effects: No substance increases in mutant frequency were observed after treatment with Benzoflex S-358

Interpretation/conclusion: It is concluded that Benzoflex S-358 did not demonstrate mutagenic potential in this *in vitro* gene mutation assay

Data Quality: 1, Reliable without restrictions

References: Benzoflex S-358. Mammalian Cell Mutation Assay. Huntingdon Life Sciences. 1998.

MAMMALIAN CHROMOSOME ABERRATION TEST

Test Substance: See boiling point for purity

Solvent: DMSO

Study Type: *In-vitro* Mammalian Chromosome Aberration Test in human lymphocytes conducted on Benzoflex S-358

Test Method: OECD 473

GLP: Yes

Year Performed: 1997

Laboratory: Huntingdon Life Sciences

Species/Strain: Human lymphocytes

Concentrations: Test 2 w/o S9 50, 100, 150, 200, 300, 400, 500, 600 ug/ml
w/S9 50, 100, 200, 400, 50-0, 600, 700, 800 ug/ml

Metabolic Activation: Sprague-Dawley rat liver

Quantity of Activator: 1.25 and 5 ml

Induction: Stimulated by Aroclor 1254

Criteria for Evaluating Results:

Aberrations were scored according to the classification of ISCN (1985). An International System for Human Cytogenetic Nomenclature, Harden, DG and Klinger, HP (Eds). S. Karger AG, Basel

Positive/Negative Controls: Positive without S9:
Mitomycin C in sterile deionized water
Positive with S9:
Cyclophosphamide in sterile deionized water
Negative DMSO

Results:

Cytotoxic concentration: With metabolic activation toxic @ 800-Test 2 800
Without metabolic activation toxic above 400-Test 1
Test 2 600

Precipitation concentration: Test 1
6.25, 12.5, 25, 50, 100, 200, 400 and 800 ug/ml
Test 2
W/S9 0 800m 400
W/O S9 600
W/S9 500-800

Genotoxic Effects: Benzoflex S-358 caused no statistically significant increases in the proportion of aberrant cells, when compared to the solvent control, in either the presence or the absence of S9 mix.

Data Quality: 1, Reliable without restrictions

References: Benzoflex S-358. *In-vitro* Mammalian Chromosome Aberration Test in human lymphocytes. Huntingdon Life Sciences. 1998.

ADDITIONAL STUDIES

Benzoflex S-358. Skin Sensitization to the Guinea Pig. Huntingdon Life Sciences. 1998. OECD 406. Benzoflex S-358 did not produce evidence of skin sensitization (delayed contact hypersensitivity) in any of twenty test animals. Evidence of skin sensitization was produced by hexyl cinnamic aldehyde (HCA) in all ten positive controls thus confirming the sensitivity of the method.

Benzoflex S-358. Acute Toxicity (LC_{50}) to the Earthworm (*Eisenia foetida).* Huntingdon Life Sciences. 1998. OECD 207. Under the conditions of this study, the LC_{50} of Benzoflex S-358 to the earthworm was found to be in excess of 1000 ppm. The NOEL was considered to be 1000 ppm.

Evaluation of Velsicol Benzoflex S-358 Plasticizer for Estrogenic Activity Using Vaginal Cornification and the Uterotrophic Response in the Ovariectomized Adult Rat as the Endpoints. BIOQUAL, Inc. 1997. Benzoflex S-358 did not induce vaginal cornification at doses of 250, 700, 1400, 2100 or 2800 mg/kg/day for 7 days by oral gavage in ovariectomized adult Spraque-Dawley (CD) rats. Clinical signs of toxicity were observed in animals dosed at 2800 mg/kg/day, with two deaths prior to the end of treatment. One animal in the 1400 mg/kg/day treatment group died prior to the end of treatment. Benzoflex S-358 did not stimulate a uterine weight increase or an increase in the uterine weight to final body weight ratio at doses of 250, 700, 1400, 2100 or 2800 mg/kg/day for 7 days. Collectively, when compared with the vehicle control and positive controls, these data demonstrated that Benzoflex S-358 did not exhibit estrogenic activity up to and including the maximally tolerated dose.